

## ***Waminoa brickneri* n. sp. (Acoela: Acoelomorpha) associated with corals in the Red Sea**

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### **Abstract**

While the majority of acoels live in marine sediments, some, usually identified as *Waminoa* sp., have been found associated with corals, living closely appressed to their external surfaces. We describe a new species collected from the stony coral *Plesiastrea laxa* in the Red Sea. *Waminoa brickneri* n. sp. can infest corals in high numbers, often forming clusters in non-overlapping arrays. It is bronze-colored, owing to the presence of two types of dinoflagellate endosymbionts, and speckled white with small scattered pigment spots. Its body is disc-shaped, highly flattened and circular in profile except for a small notch at the posterior margin where the reproductive organs lie. The male copulatory organ is poorly differentiated, but comprises a seminal vesicle weakly walled by concentrically layered muscles, and a small penis papilla with serous glands at its juncture with the male pore. The female system comprises a separate female pore, ciliated vagina, seminal bursa, 4–8 weakly sclerotized nozzles, and paired ovaries. Similarities with *Haplodiscus* spp. as well as features characteristic of the Convolutidae, including similarity in 18S rDNA sequence, warrant reassigning *Waminoa* to the Convolutidae.

**Key words:** turbellarians, endosymbionts, *Haplodiscus*

### **Introduction**

The Acoela comprises mostly free-living flatworms common in intertidal and subtidal marine environments. The group has recently garnered attention because molecular sys-

tematists have proposed that it is the most basal group of bilaterian animals (Ruiz-Trillo *et al.* 1999; Baguña *et al.* 2001). While the majority of acoels live in sediments, some have been found to be epizoic on corals in the Coral Sea (Winsor 1990), the Red Sea (Barneah *et al.* 2004), and the Micronesian region of the Pacific (Trench & Winsor 1987; Gustav Paulay, *pers. comm.*). One of these, an unidentified species from Palau, was featured on the cover of *Science* (Vol. 283, 5409, March 1999), and others from a variety of locations are featured on Web sites (for example, Zubi 2004). Such acoels also appear by happenstance in seawater aquaria harboring corals (Hendelberg & Åkesson 1988, Winsor 1990, Bartolomaeus & Balzer 1997), and, because of their potential to multiply rapidly in aquaria, aquarists refer to them as “red pest” or “plague flatworm” (Anonymous 2004; Zubi 2004). Whether the negative nature of these common names reflects that they cause real damage to corals or simply that aquarists find them unsightly is not clear.

The identities of coral-associated acoels have been established for four species. *Convolutriloba retrogemma* Hendelberg & Åkesson, 1988, and *Convolutriloba longifissura* Bartolomaeus & Balzer, 1997, were found in aquaria containing corals of unknown provenance, and *Convolutriloba hastifera* Winsor, 1990, was found in the wild on an unnamed coral from the vicinity of Townsville, Australia. The fourth species, *Waminoa litus* Winsor, 1990, also came from these Australian waters, collected on the soft coral *Sarcophyton* as well as from the stony coral *Porites* in Micronesian waters (Trench & Winsor 1987). Two other unidentified and potentially distinct species of *Waminoa* are reported (Winsor 1990) from stony corals in the same Australian waters as *Waminoa litus*.

Like the *Waminoa* species of North Queensland, Australia, a *Waminoa* species in the Red Sea is found associated with both stony and soft corals, specifically on at least 14 species of corals (Barneah *et al.* 2004). Like other undescribed species of *Waminoa* reported anecdotally, this species infests some corals in relatively large numbers and is often sufficiently conspicuous to make a coral appear spotted. We describe that species here and name it *Waminoa brickneri* in honor of its discoverer, Dr. Itzhak Brickner of Tel Aviv University, a talented coral-reef ecologist.

## Materials and methods

Sexually mature specimens were collected at Eilat, Israel, by SCUBA on 31 March 2004, from the stony coral *Plesiastrea laxa* in 8–10 meters depth near the oil jetties and from the soft coral *Stereonephthya cundabuluensis* in the reef across from the Inter University Institute. The worms were brought to the laboratory on pieces of coral removed by hammer and chisel or scissors and transported in plastic containers. In the laboratory, the worms could be dislodged from the corals with a current from a pipette. The animals were transferred to clean Petri dishes, fixed in 2.5% (v/v) glutaraldehyde in sea water, and then shipped in fixative to the laboratory in the USA for further processing. The specimens were then washed in phosphate buffer, post-fixed in phosphate-buffered 1% (v/v) osmium tetroxide, dehy-

drated in acetone, and embedded in EmBed/Araldite epoxy resin. Fixation in osmium and dehydration steps was accelerated by microwave radiation (Samsung oven, with each change of solution treated with two 7-s irradiations at 650 W separated by a 20-s hiatus; the specimen vial was kept on ice in the oven [Giberson & Demaree 1995]). Serial thick sections (2  $\mu$ m) were prepared according to Smith & Tyler (1984) and stained without deresination in toluidine blue.

For assessing affinities of the acoel by molecular-sequence characters, genomic DNA was extracted from a single specimen (from the stony coral *Plesiastrea laxa* at 12 meters depth across from the Inter University Institute in Eilat) following the protocol in the QIAGEN (2002) DNeasy™ tissue extraction kit (Qiagen, Hilden, Germany). From this, the 18S rDNA sequence was obtained using the protocol in Norén & Jondelius (1999).

## Results

### *Waminoa* Winsor, 1990

#### *Waminoa brickneri* sp. nov. (Figs. 1–3)

**Type Material.** Holotype (ZMTAU VR 25067): epoxy-embedded specimen sagittally sectioned into 2- $\mu$ m-thick sections stained with toluidine blue. Paratype (ZMTAU VR 25068): epoxy-embedded specimen sagittally sectioned into 2- $\mu$ m-thick sections stained with toluidine blue.

**Type Repository.** Zoological Museum, Tel Aviv University, Israel.

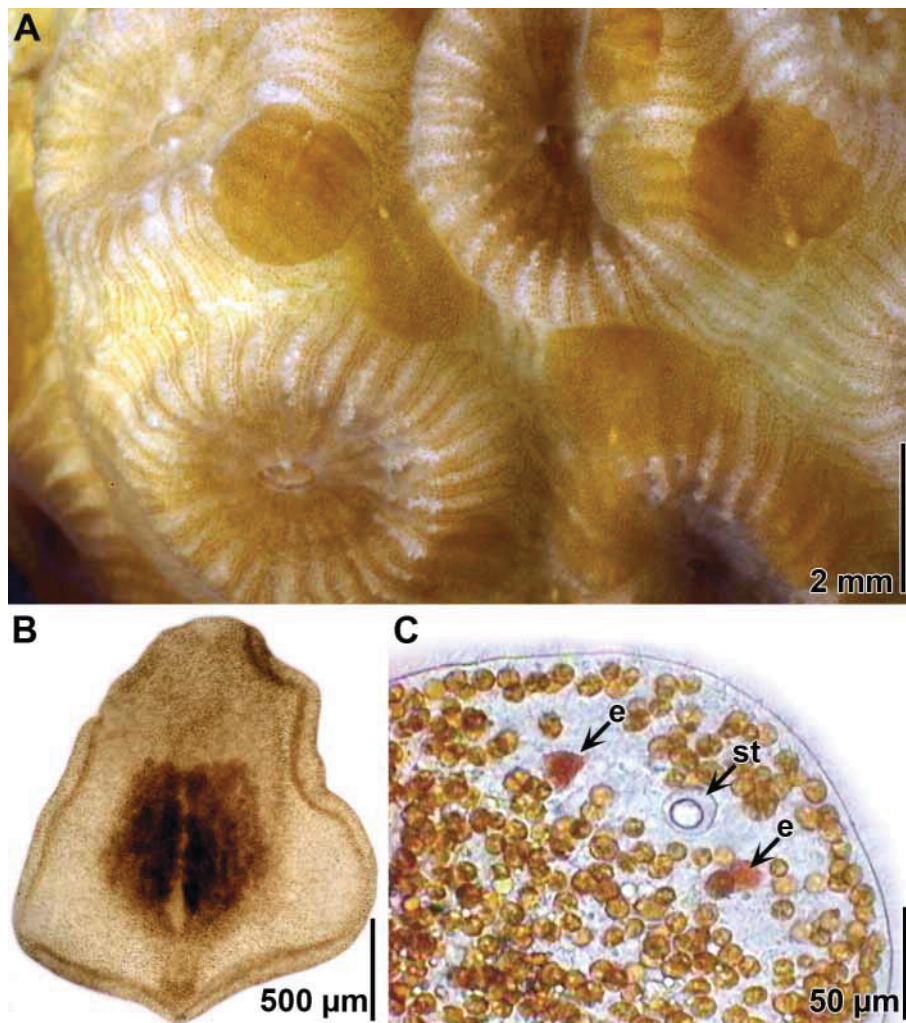
**Type Locality.** Eilat, Israel, near the oil jetties (34.93° N, 29.52° E) from the coral *Plesiastrea laxa*, 21 March 2004.

**Other Material Observed.** Serial toluidine-blue-stained sagittal sections of 4 epoxy-embedded sexually mature specimens and 3 immature specimens collected by O. B. on 21 March, 2004, and 3 June, 2003, from *Plesiastrea laxa* and *Stereonephthya cundabiluensis*, respectively.

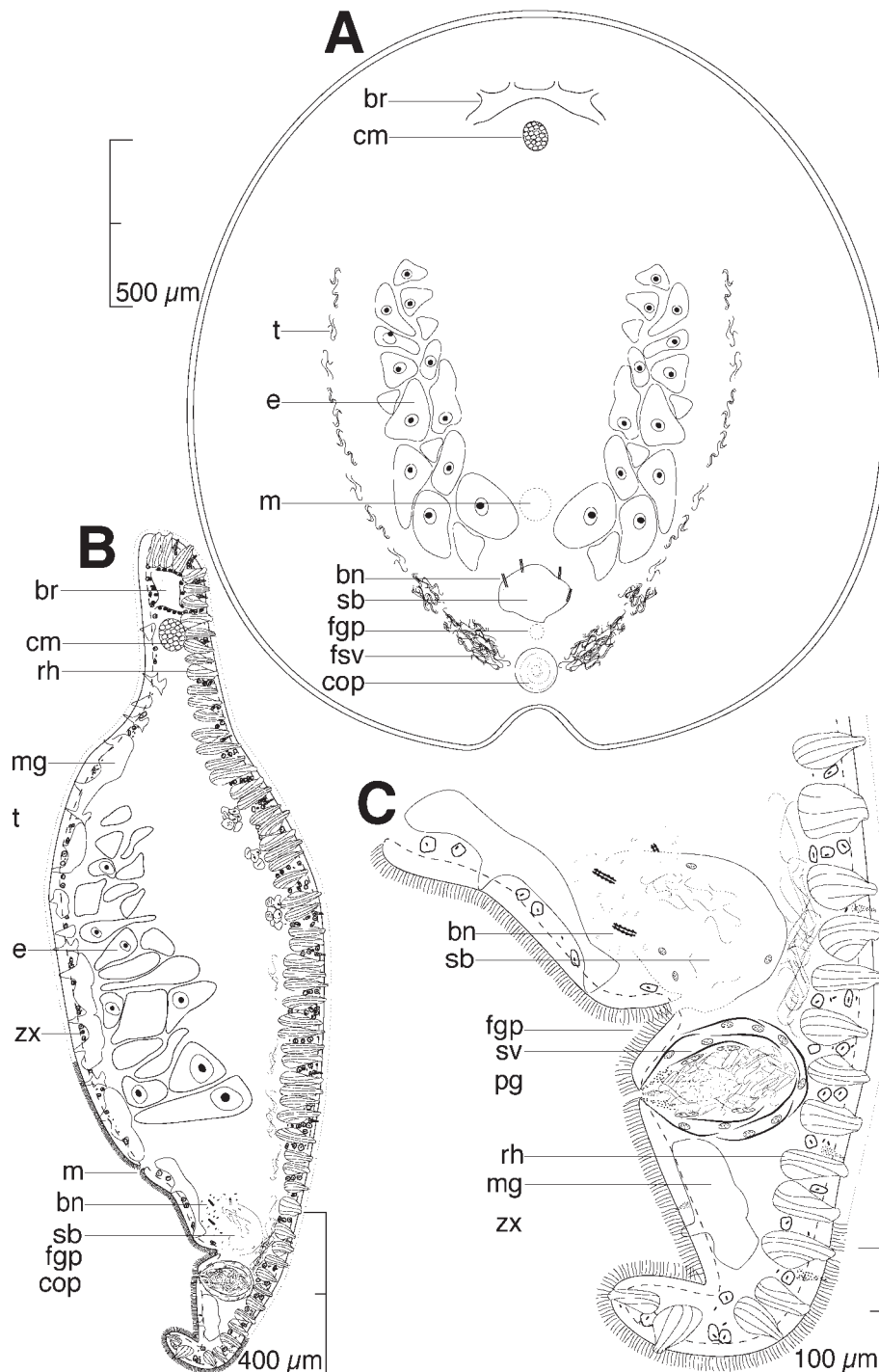
**Etymology.** Species named for Dr. Itzhak Brickner who discovered it and its symbiosis with corals.

**Description.** *Waminoa brickneri* is a flat, disc-shaped acoel, 3–4 mm in diameter and 1 mm thick, found closely appressed to the external surfaces of stony and soft corals (Fig. 1A). It occurred in groups of non-overlapping individuals causing the corals it infested, especially lightly colored ones, to appear spotted. The body was circular in profile except for a small medial notch at the posterior margin where the reproductive organs lay. The animal appeared bronze-colored with small white speckles, deriving its coloration from abundant dinoflagellate endosymbionts and scattered white pigment spots. Fixed specimens were more ovate in shape (Fig. 1B), narrowed at the anterior end and broader posteriorly, measuring 2.2–3.2 mm long, and lacked the pigment spots.

Two types of endosymbionts were harbored in the parenchyma—a more abundant smaller type measuring about 4–8  $\mu\text{m}$  and a larger type measuring about 11–16  $\mu\text{m}$  (Fig. 1C). Both types of endosymbionts lay scattered throughout the parenchyma and both were clearly identifiable as dinoflagellates by their permanently condensed chromosomes. By differences in the pyrenoid and by comparison with symbionts in *W. litus* and other acoel species, the smaller type appeared to be a species of *Symbiodinium*, while the larger one appeared *Amphidinium*-like (Barneah *et al.* 2004).

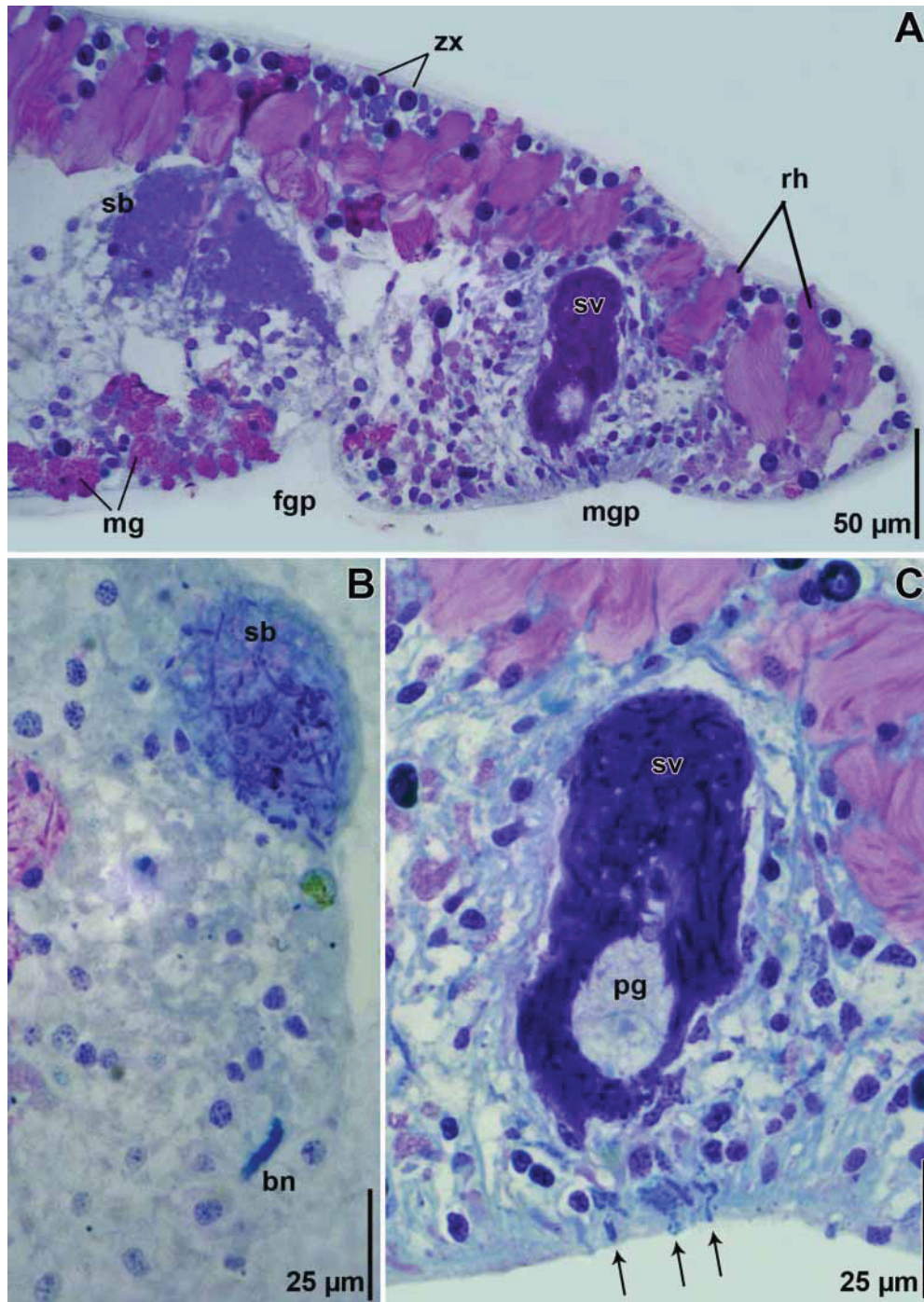


**FIGURE 1.** *Waminoa brickneri* sp. nov. A. Live animals infesting the coral *Plesiastrea laxa*. B. Ventral view of fixed adult specimen. C. Anterior end of live freshly hatched juvenile. e, eyespots; st, statocyst.



**FIGURE 2:** *Waminoa brickneri* sp. nov. Reconstructions showing arrangement of organs. A. Dorsal reconstruction of whole organism. B. Sagittal reconstruction of whole organism. C. Sagittal reconstruction of reproductive organs. bn, bursal nozzle; br, brain; cm, cell mass; cop, male copulatory apparatus; e, egg; fsv, false seminal vesicle; fgp, female gonopore; m, mouth; mg, mucous gland; pg, penis glands; rh, rhabdoid gland cell; sb, seminal bursa; sv, seminal vesicle; t, testes; zx, zooxanthellae.





**FIGURE 3:** Light micrographs of sections of *Waminoa brickneri* **sp. nov.** showing position of reproductive organs. A. Seminal bursa and male copulatory organ in mid-sagittal section. B. Tangential section of seminal bursa showing one bursal nozzle projecting caudally. C. Male copulatory organ; arrows point to serous glands that flank male gonopore. bn, bursa nozzle; fgp, female gonopore; mg, mucus glands; mgp, male gonopore; rh, rhabdoid glands; pg, penis glands; sb, seminal bursa; sv, seminal vesicle; zx, zooxanthellae.

The epidermis was generally transparent, fully ciliated, and richly glandular (Fig. 2B,C; 3A). Especially abundant were rhabdoid glands in the dorsal body wall and lateral sides. Like rhabdoid glands in other acoels, these glands had narrowed apices that gave them a tooth-like appearance in sectioned material, and their rhabdiform secretory granules appeared striate. The ventral body wall was dominated by mucous glands having more amorphous secretory bodies; these mucous glands also occurred scattered in the dorsal body wall, but no rhabdoid glands occurred on the ventral side. Both rhabdoid and mucous glands stained metachromatically with toluidine blue, indicating presence of glycosaminoglycans; the mucous glands stained more darkly red, indicating more acidic polysaccharides. A third type of gland appeared to be a serous gland containing small spherical secretion granules staining dark blue with toluidine blue. It occurred sparsely scattered in both the dorsal and ventral body walls and more abundantly supraternally at the anterior end; glands concentrated around the male gonopore appeared to be of the same type (Fig. 3C).

The mouth was ventral in the posterior third of the body length. The digestive syncytium contained no recognizable contents—specifically, no nematocysts, which would indicate feeding on the coral tissues, could be found.

Although we found freshly hatched juveniles with a statocyst and paired eyes (Fig. 1C), mature specimens of *W. brickneri* appeared to lack both. Also lacking was a frontal organ. The brain appeared to be bilobed and lay insunk just under the epidermis close behind the anterior tip of the body (Fig. 2); the two main masses consisted of central neuropile and nucleated rind and were connected by a short medial commissure. Behind it a short distance was a globular mass of unknown function consisting of close-packed, palely staining spherical cells with large nuclei.

The copulatory organs produced a slightly thickened ridge medially in the terminal quarter of the body (Fig. 1A). The seminal vesicle was most prominent, appearing white from contained sperm, and lay just in front of the posterior notch in the body margin. The seminal bursa, though unpigmented, continued the ridge anterior of the seminal vesicle, and the mouth and central digestive syncytium continued it anterior of that toward mid-body.

The paired ovaries extended from about the second quarter of the body length posteriorly behind the mouth and were ventrally situated (Fig. 2). The vagina was ciliated and opened on the ventral body wall immediately anterior to the male gonopore (Fig. 3A). It led to a seminal bursa consisting of an ill-defined syncytial mass with scattered and clumped sperm; extending from the lateral and anterior edges of the bursa were multiple sclerotized bursal nozzles. We could identify at least 4 of these small (3  $\mu$ m diameter, 12  $\mu$ m length) nozzles in sections of one specimen and at least 8 each in sections of two other specimens.

Paired testes lay dorsal and lateral to the ovaries (Fig. 2), and led posteriorly to the seminal vesicle. The seminal vesicle was walled by thin, loosely concentric muscles and

reached to an indistinct penis papilla (Fig. 3). The center of the seminal vesicle in all specimens contained a mass of light-staining tissue interpreted as penis glands. Dark-staining, small-granuled serous glands surrounded the male gonopore. Flanking the seminal vesicle were prominent false seminal vesicles continuous with the tracts of sperm descending from the testes (Fig. 2).

The partial 18S rDNA sequence of *Waminoa brickneri*, which we deposited in GenBank under accession number AJ875221, emerged as most closely related to “Acoel sp. strain Asp2” (AJ012526) and “Acoel sp. strain Asp3” (AJ012525) in a BLAST search of GenBank sequences. These two acoel species are described there as being supplied by the Coral Reef Research Foundation, Palau, and the latter one specifically as a “striped acoel, found on *Porites* coral.”

Preliminary analysis of sequence data (unpublished) showed the sequence of *W. brickneri* to group most closely to sequences of members of the family Convolutidae (e.g., *Amphiscolops* sp.), not the Haploposthiidae in which *Waminoa* is currently classified.

## Discussion

*Waminoa brickneri* is clearly closely related to *Waminoa litus*, having the same habitus (flat, generally disc-shaped body with a posterior notch), occupying the same habitat (the external surfaces of corals), having similarly simple male copulatory organs, and bilobed brain lying in front of a globular cellular mass of unknown function. It differs from *W. litus* in possessing a well-developed, muscular seminal vesicle, a more prominent seminal bursa, more than 2 bursal nozzles, and separate female and male gonopores (*W. litus* has no female gonopore, while the female pore of *W. brickneri* is prominent and opens to a ciliated vagina; its male gonopore is indistinct). The body wall of *W. brickneri* is also more richly glandular than that of *W. litus*.

Winsor (1990) erected the genus *Waminoa* for acoels he found infesting corals in northern Australian waters and placed it in the family Haploposthiidae because of the relative simplicity and position of its male copulatory organ, features characterizing that family. *Waminoa* stands out among haploposthiids by its possession of algal symbionts, eyespots, paired ovary and testis, and seminal bursa.

As Winsor (1990) himself recognized, *Waminoa* species are similar to those of *Haplodiscus* Weldon, 1888, a genus of pelagic species. Like them, *Waminoa* is disc-shaped in life and ovate or trapezoidal when fixed (*cf.* Dörjes 1970). The copulatory organs in *Haplodiscus* species are also subterminal and comprise a penis tube invaginated into a bell-shaped muscular mass that serves as a seminal vesicle (Dörjes 1970). A somewhat similar tube appears in *W. litus*, but *W. brickneri* bears an indistinct penis papilla (something Winsor [1990] also attributes to some specimens of *W. litus*). The muscular seminal-vesicle-like bell of *Haplodiscus* appears quite similar to the seminal vesicle of *W. brickneri* but does not encompass a mass of sperm as does the seminal vesicle of *W. brickneri*. A semi-



nal bursa, as occurs in the *Waminoa* species, is known only in *Haplodiscus bocki* Dörjes, 1970, but has probably been overlooked in the other *Haplodiscus* species (Dörjes 1970). *Haplodiscus bocki* also bears the peculiar anterior globular mass of cells seen in *Waminoa*; Dörjes (1970) describes this mass as surrounding the statocyst, but his drawings show the statocyst only as a space, and it may well not be here actually.

Like other convolutids, both *Waminoa* and *Haplodiscus* have relatively large body size, algal symbionts, and a pair of eyespots. These characters have been shown (Hooge & Tyler 2005) to be useful for characterizing the Convolutidae (as well as the Sagittiferidae); they are present in only one other species of the Haploposthiidae besides *Waminoa litus*, namely *Pseudohaplogonaria sutcliffei* (Hanson, 1961), another species that is likely misplaced taxonomically. Unlike most convolutids, however, *Waminoa* and *Haplodiscus* lack a tubular muscular penis and instead have a diminutive copulatory organ. Such reduction is known, however, for species of the convolutid genus *Polychoerus* Mark, 1892. In other words, *Waminoa* fits sufficiently well within the Convolutidae. This placement is corroborated, moreover, by the 18S rDNA sequence data, which as been shown to be an effective means of determining the phylogenetic relationships of acoels (Hooge *et al.* 2002).

We therefore transfer the genus *Waminoa* to the family Convolutidae, and offer the following emended diagnosis:

#### Convolutidae Graff, 1905

##### *Waminoa* Winsor, 1990 (emend)

**Diagnosis.** Convolutidae discoid to obcordate in shape; color results from pigments and symbiotic algae; often with two coexisting species of algal symbionts; brain insunk; mouth ventral and in posterior third of body; rhabdoids present; ovaries and testes paired; with sub-terminal male genital pore. Male copulatory organ composed of muscular seminal vesicle filled with sperm. Female gonopore present or absent. Seminal bursa with 2 or more (2–8) bursal nozzles. Statocyst and eyes absent in mature forms, but present in immature specimens. Frontal organ absent. Epizoic on corals.

**Type species.** *Waminoa litus* Winsor, 1990

**Remarks.** Whether either of the two *Waminoa* species are synonymous with species of *Haplodiscus* remains a possibility. Most of these *Haplodiscus* species were described from only a paucity of specimens collected in plankton tows and, except for *H. piger*, not observed alive (Dörjes 1970). Clarifying the relationship between *Haplodiscus* and *Waminoa* awaits finding more live material of both genera.

The two algal symbionts in *Waminoa brickneri* are species of *Symbiodinium* and an *Amphidinium*-like dinoflagellate and reside intracellularly within parenchymal cells. Like other convolutids, *W. brickneri* probably does not rely solely on its symbionts for nutrition. It does not appear to consume coral tissue, however, in that no nematocysts occur in its digestive tissue.

Coral-infesting acoels like *Waminoa brickneri* are likely to be widespread, occurring wherever there are shallow tropical reefs. Besides the published reports of occurrence in the Coral Sea, the Red Sea, and Micronesia, anecdotal reports on reef-animal Web sites as well as some unpublished reports of it in waters off Kenya and Tanzania (Yehuda Benayahu *personal comm.*) and Indonesia (Gustav Paulay *personal comm.*) show distribution in the Indo-Pacific region. As Trench & Winsor (1987) suggested for *Waminoa*, its coral-infesting form may be a benthic phase in a life cycle involving also pelagic individuals, which would be identified as species of *Haplodiscus*. Occurrence of *Haplodiscus* species in the Bahamas, the Gulf Stream, the Sargasso, the North Equatorial Current, and the Gulf of Naples (see distribution records and literature in Tyler *et al.* 2005) hint at occurrence of coral-associated acoels in the Atlantic Ocean and Mediterranean Sea as well.

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